



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: 12/07/2005

SUBJECT: **Apiguard (Thymol):** Addendum to the Review of Response to Deficiency Letter, Waiver Rationales, and Product Chemistry

DP Bar Code #:316472, EPA File Symbol Number: 79671-R, Decision #341453
MRIDs: 465245-01, 465245-02, 464856-01, administrative material with
embedded waivers

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THRU: Russell Jones, Senior Biologist /s/ 12/07/05
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TO: Andrew Bryceland, Regulatory Action Leader
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ACTION REQUESTED: Vita (Europe) Limited in c/o Landis International, Inc. has submitted a petition for exemption from the requirements of a tolerance for the use of thymol in bee hives. The food commodities affected by the proposed use are honey and wax. Supporting documents have been provided by the registrant and EPA. Thymol is the active ingredient in Apiguard, an acaricide. The proposed application of Apiguard to the inside of bee hives is made to decrease the incidence of Varroa mite infestation.

SUMMARY RECOMMENDATIONS:

- 1) The toxicology database for thymol and Apiguard are complete and no new information needs to be considered.
- 2) The residue data for the use of Apiguard in bee hives is sufficient and no new information need be submitted. Thymol residues resulting from the application of Apiguard are expected to be below levels of concern 30 days following exposure. Dietary risks from the consumption of thymol residues in honey do not significantly increase the exposure to thymol from other dietary commodities.

Dietary exposure to thymol residues in wax is not expected to be significant.

- 3) Risks from consumption of thymol-contaminated water resulting from application in bee hives is not anticipated.
- 4) The proposed exemption for the requirement of a tolerance is supported by the studies/data/waiver requests submitted by the registrant and by supplemental information supplied by EPA. It is highly unlikely that there will be any adverse effects to humans from the consumption of honey, wax, or water that has been contaminated with thymol from proposed applications of 100 g Apiguard gel/year (25 g thymol).

SUPPLEMENTAL SUMMARY RECOMMENDATIONS:

1) A previous memo (Carlson, 07/19/05) requested information on the physical, chemical, and toxicological similarities of [REDACTED] in relation to [REDACTED]. This information was requested in order to bridge from acute toxicity data generated using [REDACTED] to the new formulation containing [REDACTED]. Physical, chemical, and toxicological information on [REDACTED] were subsequently provided to EPA by the registrant via email [REDACTED]

[REDACTED] Toxicology, regulatory, health, safety & environmental studies of [REDACTED]

Acute toxicity studies using [REDACTED] (MRIDs 46043504 - 46043509) are **ACCEPTABLE**, and conform to guideline study recommendations (Sjoglad DERs, 09/16/04). The similarity of [REDACTED] in physical, chemical, and toxicological properties allows bridging of the acute toxicology data from [REDACTED]. The acute toxicity studies using [REDACTED] therefore, support the respective data requirements for the EP containing [REDACTED]

2) A previous memo (Carlson, 07/19/05) determined that submitted analytical studies (MRIDs 46524501 and 46524502) were unacceptable. EPA has supplemented the application with information communicated by the registrant. **Information submitted by the registrant has upgraded both of the preliminary analyses (MRIDs 46524501 and 46524502) to ACCEPTABLE.** The information presented by EPA and the registrant is presented below.

- A) The expiration dates for batches of Apiguard tested have been reported as 24 months for a 50g tray and 18 months for a 3 kg tub, with a maximum storage temperature of 30°C (MR46043503, MSDS dated November 15th, 2001).

- B) The registrant explained discrepancies between handwritten batch notes and the final certificates of analyses as reported in Table 1 of the review DER. Results displayed on batch notes are in-process samples, whereas those displayed on the Certificate of Analysis are from samples taken following manufacture (Watkins, 08/15/05).
- C) The registrant explained that the methodological discrepancies between manufacturing processes and handwritten notes were the result of transcription errors by the operator. The batch manufacturing protocol has been subsequently reworded to address these issues (Watkins, 08/15/05).

3) A public comment has been received objecting to "any tolerance, exemption, or waiver allowing more than zero residue [of thymol] on food" (B. Sachau, 2005). This objection was supported by the arguments that A) embryonic chickens have multiple malformations following thymol injection into the yolk or air sac, and B) Switzerland has established an Maximum Residue Limit (MRL) of 0.8mg/kg.

In response to these comments, EPA acknowledges that these arguments are factual in nature and do exist in peer-reviewed and regulatory literature. The relevance of these articles in setting a zero concentration tolerance, however, is questionable.

Currently, EPA does not use chickens (or intrayolk or intra-airsac exposure routes) as an animal model for developmental toxicity because of the inconsistencies in developmental physiology and anatomy between the two species. Developmental timing, duration, and potential environmental effects on developing young are also different in mammals and birds, again precluding this model for use in setting developmental toxicity endpoints for the regulation of pesticides.

The results from the chicken study, although interesting, are of questionable relevance to mammals. Developmental malformations have not been found following thymol exposure to other mammalian species such as mice, rats, hamsters, and rabbits (Environmental Risk Management Agency of New Zealand, 2005). In addition, Mortazavi et al. (2003) reported no external tissue abnormalities in fetuses following dosing of female rats with an infusion of the plant *Satureja khuzestanica* (which has the components thymol and carvacrol).

Regulatory limits have been set for thymol in other countries. The Swiss Federal Department of the Interior has set a tolerance (MRL) concentration for thymol in honey as an antiparasitic agent (0.8 mg/kg; pharmacological substance active in nutrition or therapeutic application; 817.021.23). This tolerance was derived to prevent exceedance of the *taste* threshold for thymol in honey (1.1 - 1.3 mg/kg; Bogdanov et al., 1999), not safety. Tolerances set by EPA are based on "the reasonable certainty of no harm" and therefore, are not constrained by criteria such as taste.

BACKGROUND: see previous memo (Carlson, 07/19/05) and below.

Thymol is a volatile essential oil that is extracted from various species of plants such as thyme (0 - 60% thymol) and mandarin and tangerine oils (0.1-0.03%). Thymol is used for flavoring in food and beverages (5 - 78 mg/kg) and has been quantified in candy (9.4 mg/kg), ice cream (44 mg/kg), and chewing gum (100 mg/kg).

Thymol is currently approved by the U.S. Food and Drug Administration (FDA) as a synthetic flavoring substance for the direct addition to food for human consumption (FDA, 21CFR §172.515) and as a preservative and indirect food additive of adhesives (FDA, 21CFR §175.105). The source plant of thymol, thyme or wild and creeping thyme (*Thymus vulgaris* L., or *Thymus serpyllum* L.) is also currently acknowledged by FDA as a spice, natural oil, oleoresin, or natural extract that is generally recognized as safe (21CFR §182.10, 21CFR §182.20). Although constituents vary depending on species and cultivation method, thyme can be comprised of up to 60% thymol (De Vincenzi et al., 1991).

In contrast to food uses, FDA has determined that there is inadequate evidence to establish the (general recognition of) safety and effectiveness for thymol when used as a topical acne treatment; a nasal decongestant; a dandruff/seborrheic dermatitis/psoriasis treatment; an external analgesic or anesthetic; a fever blister and cold sore treatment; a poison ivy, oak, and sumac treatment; an oral health care treatment; a skin protectant-astringent treatment, and a topical antifungal treatment (FDA, 21CFR §310.545). FDA has also banned it from use as an ingredient in smoking deterrent products (FDA, 21CFR §310.544), and an over the counter treatment for boils (FDA, 21CFR §310.531) because these uses have not been shown to be safe and effective.

Regulatory limits have been set for thymol in other countries. The Swiss Federal Department of the Interior has set a tolerance value for thymol in honey as an antiparasitic agent (0.8 mg/kg; pharmacological substance active in nutrition or therapeutic application; 817.021.23). This tolerance was derived to prevent exceedance of the taste threshold for thymol in honey (1.1 - 1.3 mg/kg; Bogdanov et al., 1999). The European Agency for the Evaluation of Medicinal Products (Committee for Veterinary Medicinal Products; EMEA/MRL/075/96) has also established an indication of use for thymol at 10 mg per animal (horse, swine, cattle, sheep, and dogs) for up to 5 days for the treatment of respiratory tract ailments. Residues of thymol in treated animals and products were not thought to be of toxicological concerns for humans in this case. The Committee of Experts on Flavouring Substances of the Council of Europe has also established a limit for thymol inclusion in food at 50 mg/kg and beverages at 10 mg/kg (2002). Thymol is currently exempted from a food MRL in New Zealand.

Four chemicals are structurally similar to thymol (p-cymene, carvacrol [o-thymol], p-thymol [o-Cymen-5-ol], and o-thymol [Phenol, 2-isopropyl-6-methyl]) and differ only in hydroxylation of the aromatic ring. P-cymene lacks hydroxylation at any aromatic sites while carvacrol and o-thymol have a hydroxyl group on a site on the aromatic ring adjacent to the methyl group (ortho position) and that of thymol (Austgulen et al., 1987; Walde et al., 1983). Geometric similarity of the parent chemicals, similar breakdown metabolites, and the ability for the parent compounds and metabolites to metabolically interconvert ensure that physico-chemical and toxicological information for these chemicals will be approximately similar. These similar compounds are also utilized to supplement previously provided information.

REGULATORY HISTORY: see previous memo (Carlson, 07/19/05) and below.

On August 17th of 2005, EPA completed review of two product chemistry studies.

On August 19th of 2005, EPA completed a review memo outlining historic data gaps and study reviews for both the TGAI and EP, Apiguard.

On September 8th of 2005, EPA completed a re-review of additional information on the EP product Apiguard and the TGAI thymol.

On December 2nd of 2005, EPA completed the final review of supplemental information associated with the TGAI thymol.

DATA REQUIREMENTS FOR THE EP, APIGUARD

- 1) The toxicology database for Apiguard is complete and no new information needs to be considered.
- 2) The residue data determining the concentration of thymol in bee honey and wax following the use of Apiguard in hives is ACCEPTABLE and no new information need be submitted. Consumption of thymol residues in honey resulting from the label use of Apiguard is not expected to result in adverse effects.

Data Table: Data requirements and relevant results for the EP Apiguard can be seen in Table 1.

| TABLE 1. Physical and Chemical Properties for EP (Apiguard or Apiguard (EZ-3 formulation)) | | |
|---|--|---|
| Guideline Reference No./Property | Description of Result | Methods/Source |
| Physical and Chemical Requirements | | |
| 830.6302 Color | Opalescent, colorless to pink | Visual inspection; MRID 46043502 |
| 830.6303 Physical State | Gel, Aqueous granular gel | Visual inspection; MRID 46043502; MSDS dated November 15 th , 2001 |
| 830.6304 Odor | Strongly aromatic of thyme | Olfactory inspection; MRID 46043502 |
| 830.6313 Stability | Not required for EP stable-but avoid heating | Not required for EP; MRID 46043502; MSDS dated November 15 th , 2001 |
| 830.6314 Oxidation/Reduction: | None of the components is an oxidizer or reducer | Not appropriate for this EP |
| 830.6315 Flammability | No combustible liquids in formulation; flashpoint >100°C | Not appropriate for this EP; MSDS dated November 15 th , 2001 |
| 830.6316 Explodability | Not potentially explosive | Not appropriate for this EP |
| 830.6317 Storage Stability | Storage stability 22 months at 22 and 30°C | MRID 46043503 |
| 830.6319 Miscibility | Not an emulsifiable liquid to be diluted with petroleum solvents | Not appropriate for this EP |
| 830.6320 Corrosion Characteristics | Non-corrosive | MRID 46043503 |
| 830.6321 Dielectric Breakdown Voltage | Not to be used around electrical equipment | Not appropriate for this EP |
| 830.7000 pH | 6.5 - 8.0 | MRIDs 46043503 |
| 830.7100 Viscosity | 70,000 - 130,000 cps @ 22 and 30°C | MRIDs 46043503 |
| 830.7200 Melting Range | Not required for EP | Not required for EP |
| 830.7220 Boiling Range | Not required for EP | Not required for EP |

| Guideline Reference No./Property | Description of Result | Methods/Source |
|---|--|---|
| 830.7300 Bulk Density | Specific gravity: 1.01 g/cm ³ ; 1.005-1.025 g/cm ³ | MRID 46043502; MSDS dated November 15 th , 2001 |
| 830.7370 Dissociation Constant | Not required for EP | Not required for EP |
| 830.7550 Partition Coefficient | Not required for EP | Not required for EP |
| 830.7840 Water Solubility | Not required for EP; 77% (w/w) | Not required for EP; MRID 46043502; MSDS dated November 15 th , 2001 |
| 830.7950 Vapor Pressure | Not required for EP; 2.25*10 ³ Pa @ 20°C | Not required for EP; MSDS dated November 15 th , 2001 |
| Product Analysis Requirements | | |
| 830.1550 Product Identity | Thymol (25.00 %), [REDACTED] | MRIDs 46043501, 46198201, 46524501, -02 |
| 830.1620 Manufacturing Process | [REDACTED] | MRIDs 46524501, -02, 46198201 |
| 830.1670 Discussion of Formation of Unintentional Ingredients | No impurities of toxicological significance associated with the active ingredient are expected | MRIDs 46524501, -02, 46198201 |
| 830.1700 Analysis of Samples | 25.15 - 25.56% thymol | MRIDs 46524501, -02, 46043503 |
| 830.1750 Certification of Limits | 25 ± 3% (24.25 - 25.75 %) | MRIDs 46524501, -02, 46198201, 46043503 |
| 830.1800 Analytical Methods | Gas Liquid Chromatography | MRIDs 46043501, 46485601 |
| ¹ Residue Data Requirements | | |
| 860.1500 Magnitude of the Residue | Maximum thymol residues of 2.59 mg/kg in honey and 97.6 mg/kg in wax 30 days after treatment finish in U.S. trials; Maximum thymol residue of 4.61 mg/kg in honey and 51.6 mg/kg in wax 2 days after treatment finish in European trials; ACCEPTABLE | MRIDs 46043510 through -13 |
| ¹ Toxicology Data Requirements | | |
| 870.1100 Acute Oral Toxicity | Oral LD50>2000mg/kg, Toxicity Category III; ACCEPTABLE | MRID 46043504 |
| 870.1200 Acute Dermal Toxicity | Dermal LD50>2000mg/kg, Toxicity Category III; ACCEPTABLE | MRID 46043505 |
| 870.1300 Acute Inhalation Toxicity | Unable to produce a suitable atmosphere for inhalation testing of gel product; Toxicity Category III; ACCEPTABLE | MRID 46043506 |

Inert ingredient information may be entitled to confidential treatment
Manufacturing process information may be entitled to confidential treatment

| Guideline Reference No./Property | Description of Result | Methods/Source |
|--|---|-----------------------------------|
| 870.2400 Primary Eye Irritation | Corneal opacity >28 days, resolving iritis and conjunctival irritation, Toxicity Category I; ACCEPTABLE | MRID 46043507 |
| 870.2500 Primary Dermal Irritation | Slight to well defined erythema and slight to moderate edema clearing by day 14, Toxicity Category IV; ACCEPTABLE | MRID 46043508 |
| 870.2600 Hypersensitivity | Not a sensitizer; ACCEPTABLE | MRID 46043509 |
| Hypersensitivity Incidents | <i>Required for EP</i> | <i>Incidents must be reported</i> |
| 870.5500 Genotoxicity | <i>Not required for EP</i> | <i>Not required for EP</i> |
| 870.3550 Immune Response | <i>Not required for EP</i> | <i>Not required for EP</i> |
| 870.3100 90-day Feeding (1 spp) | <i>Not required for EP</i> | <i>Not required for EP</i> |
| 870.3250 90-day Dermal (1 spp) | <i>Not required for EP</i> | <i>Not required for EP</i> |
| 870.3465 90-day Inhalation (1 spp) | <i>Not required for EP</i> | <i>Not required for EP</i> |
| 870.3700 Teratogenicity (1 spp) | <i>Not required for EP</i> | <i>Not required for EP</i> |
| Nontarget Organism, Fate, and Expression Requirements | | |
| 850.2100 Avian Acute Oral | <i>Not required for EP</i> | <i>Not required for EP</i> |
| 850.2200 Avian Dietary | <i>Not required for EP</i> | <i>Not required for EP</i> |
| 850.1075 Freshwater Fish LC50 | <i>Not required for EP</i> | <i>Not required for EP</i> |
| 850.1010 Freshwater Invertebrate LC50 | <i>Not required for EP</i> | <i>Not required for EP</i> |
| 850.4100 Nontarget Plant Studies | <i>Not required for EP</i> | <i>Not required for EP</i> |
| 850.4350 Nontarget Insect Studies | <i>Not required for EP</i> | <i>Not required for EP</i> |

¹ Testing was done with previous formulations of Apiguard (using [REDACTED])

Note to RAL: Maximum residue concentrations of 2.59 mg/kg thymol in honey (at 30 days following treatment in U.S. trials) and 4.61 mg/kg thymol in honey (at 2 days following treatment in European trials) are above the threshold for taste, which has been established at 1.1 - 1.3 mg/kg. These are also above the Swiss MRL of 0.8 mg/kg.

Inert ingredient information may be entitled to confidential treatment

DATA REQUIREMENTS FOR THE TGAI, THYMOL

A previous memo (Carlson, 07/19/05) requested information (a registered source or physical, chemical, and Tier I toxicology information) on the TGAI, thymol. The registrant has provided information on thymol analysis, manufacturing method, and the formation of impurities (MRIDs 46485601 and 46664001). EPA has supplemented this request with publicly available information in order to facilitate information compilation and interpretation. The supplemental information retrieved by EPA and used for waiver rationales for the physico-chemical properties of the TGAI is based upon information presented in numerous sources such as: NIH Toxnet, MSDS sheets, National Library of Medicine SIS ChemiID Plus, and EPA (2001)

Physico-chemical and toxicology data from four chemicals structurally similar to thymol (p-cymene, carvacrol [o-thymol], p-thymol [o-Cymen-5-ol], and o-thymol [Phenol, 2-isopropyl-6-methyl]) were also utilized to supplement information previously provided. All compounds differ from thymol only in hydroxylation of the aromatic ring. P-cymene lacks hydroxylation at any aromatic sites while carvacrol and o-thymol have a hydroxyl group on a site on the aromatic ring adjacent to the methyl group (ortho position) and that of thymol (Austgulen et al., 1987; Walde et al., 1983). Geometric similarity of the parent chemicals, similar breakdown metabolites, and the ability for the parent compounds and metabolites to metabolically interconvert ensure that physico-chemical and toxicological information for these chemicals will be similar.

Data Guidelines 830.6302 (color), 830.6303 (physical state), 830.6304 (odor), 830.6313 (stability), 830.7000 (pH), 830.7200 (melting range), 830.7220 (boiling range), 830.7300 (bulk density), 830.7370 (dissociation constant), 830.7550 (partition coefficient), 830.7840 (water solubility), and 830.7950 (vapor pressure)

Specifically, thymol is a colorless to white (MRID 46485601) crystalline solid (MRID 46485601) that smells like thyme (NOAA MSDS), is stable under ordinary conditions of use and storage (NOAA MSDS), has a neutral pH in alcohol (National Library of Medicine SIS ChemiID Plus), is not an oxidizer or reducer, melts at 48 - 51.5°C (MRID 46485601, MSDS dated November 15th 2001, National Library of Medicine SIS ChemiID Plus, NOAA MSDS), boils at 232.5-233°C (MSDS dated November 15th 2001, National Library of Medicine SIS ChemiID Plus, NOAA MSDS), has a specific gravity of 0.97 @ 25°C/4°C (NOAA MSDS), a dissociation constant of 10.62 @ 20°C (National Library of Medicine SIS ChemiID Plus), a partition coefficient of 3.3-3.34 (Log Kow; MSDS dated November 15th 2001, National Library of Medicine SIS ChemiID Plus, EPA 2001), water solubility of 900mg/L @ 20°C or 0.1g/100g water @ 25°C (National Library of Medicine SIS ChemiID Plus, NOAA MSDS), and vapor pressure of 0.0022 mm Hg @ 25°C, 12.7 Pa @ 40°C, and 1mm Hg @ 64°C (National Library of Medicine SIS ChemiID Plus, NIH Toxnet, NOAA MSDS).

A majority of this information is not in the format of studies typically required, nor presented in scientific detail enough to be reviewed critically. Citation of this information by federal agencies and multiple sources of information with similar values, however, suggests that the information is sound and empirically derived. The physico-chemical information is therefore **ACCEPTABLE**, and fulfills its respective guidelines as presented in Table 2.

Data Guidelines 830.1620 (manufacturing process), 830.1670 (formation of

unintentional ingredients), and 830.1700 (analysis of samples)

The EPA Registration Eligibility Document (RED) for thymol (1993) lists generic data requirements including technical chemistry data for the analysis and certification of product ingredients, a copy of USP analysis with methodological clarification (if food/USP grade), physical and chemical characteristics from MSDS sheets, and a CSF supported by analytical data. The registrant has provided an **ACCEPTABLE** manufacturing process, formation of unintentional ingredients, and analytical results for thymol (MRIDs 46664001, 46485601). In brief, [REDACTED]

Data Guideline 870.1100 (acute oral toxicity)

The waiver rationale for acute oral toxicity (870.1100) is based on oral LD₅₀s from peer-reviewed publications.

The oral LD₅₀ of thymol (5-methyl-(methylethyl) phenol; CAS# 89838) has been reported in ERMA (2005) and Sax (1984) to be 980, 640-1800, and 880 mg/kg in rats, mice, and guinea pigs respectively.

The oral LD₅₀ of o-thymol (carvacrol; CAS# 499752) in rats has been reported as 810 mg/kg (FCTXAV, 1964). Following dosing rats with o-thymol, the authors observed somnolence and convulsions.

The oral LD₅₀ of p-thymol (o-Cymen-5-ol; CAS# 3228022) in mice has been reported as 6280 mg/kg (OSDIAF, 1956). Following dosing with p-thymol, the authors observed spastic paralysis with or without sensory change and respiratory stimulation.

The oral LD₅₀ of p-cymene (CAS 99876) in rats has been reported as 4750 mg/kg (Aldrich Chemical Co. Inc MSDS).

The lowest reported oral LD₅₀ concentration for thymol (640 mg/kg in mice) was chosen to determine the acute oral Toxicity Category. This concentration places thymol conservatively into **Toxicity Category III** for acute oral effects.

Data Guideline 870.1200 (acute dermal toxicity)

The waiver rationale for acute dermal toxicity is based upon information from the U.S. Environmental Protection Agencies document entitled "Risk Assessment Guidance for Superfund Volume I: Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment" and Oral LD₅₀s from laboratory testing. The guidance for dermal risk reports that dermal absorption of thymol is 61% of the absorption from the oral route (Exhibit B-3; shower scenario versus ingestion). Applying this factor to previously reported oral LD₅₀s for thymol yields dermal LD₅₀s of approximately 1607 (rat), 1049 (mouse), 1443 (guinea pig), 1229 (rabbit), and 1327 mg/kg (for the chemical isomer of thymol carvacrol in rats). These calculated

dermal LD₅₀s are similar to that (>2000mg/kg) reported by the Environmental Risk Management Agency (ERMA, 2005) of New Zealand and Anonymous (2000). The lowest calculated dermal LD₅₀ concentration for thymol (1049 mg/kg in mice) was chosen to determine the acute dermal Toxicity Category. This concentration places thymol conservatively into **Toxicity Category II** for acute dermal effects.

Data Guideline 870.1300 (acute inhalation toxicity)

The waiver rationale for acute inhalation toxicity is based upon information from the U.S. Food and Drug Administration Center for Drug Evaluation and Research and other peer reviewed publications. Thymol is added to the anesthetic halothane as a preservative (0.01%) and is considered inactive (by FDA) at this concentration (FDA, pers. commun.). Halothane is used to anesthetize dogs, cats, and other non-food animals for periods sometimes exceeding 4 hours. Anesthetic induction concentrations can typically reach approximately 5%. Calculation of the exposure from these factors yields a thymol atmospheric concentration of 5mg/L, at which permanent pathological effects on the anesthetized patients are not expected. Since this theoretical concentration is greater than 2 mg/L (the lower limit for Toxicity Category IV) thymol can be conservatively placed into **Toxicity Category IV** for acute inhalation toxicity.

Note to RAL: It should be noted that hepatitis and pulmonary edema have been reported in public literature following exposure to halothane/ thymol anesthetics (Euerby and Walker, 1984; Hutter, 1995; Hutter and Liang, 1993). Cases involving pulmonary edema have been correlated to increased thymol (16-22x normal concentrations) that had collected in anesthesia vaporizers (Euerby and Walker, 1984). Halothane/thymol has also been implicated in some hepatitis cases following anesthesia (Hutter, 1995; Hutter and Liang, 1993). Others (Smith and Reynard, 1991), however, attribute halothane/thymol hepatitis to the formation of dechlorinated halothane free radical haptens which bind to the liver and stimulate an immune response. Both hepatitis and pulmonary edema are not thought to occur at concentrations potentially encountered following the application of Apiguard (25% thymol). These reports, therefore, are largely informational in nature and do not affect the Toxicity Category of thymol.

Data Guideline 870.2400 (primary eye irritation)

The waiver rationale for primary eye irritation is based upon information presented in the Federal Register (2003) and ERMA (2005) which state that thymol is corrosive to the eyes. As described in the data guidelines and the 40 CFR §156.62, pesticides with corrosive effects to the eye are categorized into **Toxicity Category I**.

Data Guideline 870.2500 (primary dermal irritation)

The waiver rationale for primary dermal irritation is based upon information presented in the Federal Register (2003), ERMA (2005), and Barratt (1996) which state that thymol is corrosive to the skin. As described in the data guidelines and the 40 CFR §156.62, pesticides with corrosive effects to the skin are categorized into **Toxicity Category I**.

Data Guideline 870.2600 (skin hypersensitivity)

The waiver rationale for skin hypersensitivity is based on information presented in Hostynek and Magee (1997). Using quantitative structure activity relationships, Hostynek and Magee predicted that thymol is a dermal sensitizer. These results contrast that previously reported in the Federal Register (2003), Anonymous (2000), and ERMA (2005).

Note to RAL: Supplementary case studies illustrating occupational and consumer exposure have also reported that exposure to thyme dust (Golec et al., 2004; Spiewak et al., 2001), reaction products of thymol and triazine derivatives (Smeenk et al., 1987), thymol in Listerine® (Fisher, 1989), and thymol in chloroform (Lorenzi et al., 1995) can induce allergenicity. This information will not be used in the support of this registration, but can be acknowledged scientifically.

Data Guideline 870.3100, 870.3250, 870.3465 (90-day oral, dermal and inhalation)

Oral subchronic studies are typically required when the pesticidal use requires a tolerance or an exemption from the requirement of a tolerance, a food additive regulation, or its use results in repeated human oral exposure. Subacute and chronic studies submitted on September 18th of 2003 (MRIDs 46282803 and -04), presumably as waiver rationales for subchronic toxicity (870.3100), and other rationales presented by EPA **support the respective data requirement for the TGAI**. Sole support was not justified for the submitted study because certain critical aspects of the subchronic study were not reported or performed.

Although the use of thymol requires a tolerance or exemption from the requirement of a tolerance, its use as a food additive has already been established. Thymol is currently approved by the U.S. Food and Drug Administration (FDA) as a synthetic flavoring substance for the direct addition to food for human consumption (FDA, 21CFR §172.515) and as a preservative and indirect food additive of adhesives (FDA, 21CFR §175.105). The source plant of thymol, thyme or wild and creeping thyme (*Thymus vulgaris* L., or *Thymus serpyllum* L.) is also currently acknowledged by FDA as a spice, natural oil, oleoresin, or natural extract that is generally recognized as safe (21CFR §182.10, 21CFR §182.20). Although constituents vary depending on species and cultivation method, thyme can be comprised of up to 60% thymol (De Vincenzi et al., 1991).

The use pattern for Apiguard (25% thymol) precludes subchronic (daily, intermediate-term) occupational exposure. Only two applications of Apiguard, separated by 2 weeks, are made per hive. Applications are synchronized within apiaries to prohibit repopulation of untreated or previously treated hives with Varroa mites. In addition, placement within hives further mitigates exposure. Therefore, it is anticipated that occupational exposures will be of acute duration only and not subchronic.

Dietary subchronic exposure to thymol in honey is probable. Thymol residues occur naturally in some forms of lime honey (0.02-0.16 mg/kg) and thymol residues in honey (maximum 0.5 mg/kg) have been reported to be present 6 months following thymol treatment in European field studies spanning 5 years.

Thymol residues are found in other food stuffs at significantly higher concentrations than those resulting from pesticidal treatments. Thymol has been monitored in ice cream (44 mg/kg), non-alcoholic beverages (2.5-11 mg/kg), candy (9.4 mg/kg), baked goods (5.0-6.5 mg/kg), and

chewing gum (100 mg/kg). It is also a constituent in cooking herbs derived from wild or creeping thyme (up to 60%) and mandarin and tangerine oils (0.1-0.03%). Because the dietary contribution of thymol from honey is expected to be negligible compared to that already in the diet, subchronic studies are not required.

This waiver rationale for 90-day feeding (870.3100), 90-day dermal toxicity (870.3250), and 90-day inhalation toxicity (870.3465) studies, therefore, supports the respective data requirements for the TGAI.

Data Guideline 870.5000 (genotoxicity and mutagenicity)

Genotoxicity and mutagenicity studies submitted on September 18th of 2003 (MRIDs 46282801 and -02), presumably as waiver rationales for genotoxicity (870.5000) and other peer-reviewed publications retrieved by EPA, **support the respective data requirement for the TGAI.**

Thymol has been reported to be non-mutagenic in multiple Ames tests (strains TA97, TA98, and TA100 w and w/out metabolic transformation with S9 incubation (Azizan and Blevins, 1995; MRID 46282801; Stamatii et al., 1999), but positive in unscheduled DNA synthesis (liquid scintillation), sister chromatid exchange, and cell transformation tests in Syrian hamster embryo cells in culture (Zani et al., 1991, MRID 46282802; ERMA, 2005; Tsutsui, 1987). In addition, thymol does not induce chromosomal aberrations in *Allium cepa* (Grant, 1982)

The chemical isomer of thymol, carvacrol, does not form sister chromatid exchanges in *in vitro* assays with human peripheral blood lymphocytes and inhibits SCE induction by mitomycin C (Ipek et al., 2003).

Steam distilled extracts of three species of *Thymus* (*capitatus*, *citriodorus*, *vulgaris*) also were negative for DNA damaging activity and mutagenicity in the Ames test (strains TA1535, TA1537, TA98, and TA100 with and w/out metabolic activation). They were also non-mutagenic in a salmonella/microsome assay, did not induce the formation of micronuclei in mice, even when orally dosed in the toxic range (1100 mg/kg bw). Further, in the A/He strain of mice, thymol did not increase the incidence of spontaneous lung tumors following repeated intraperitoneal dosing (Anonymous, 2000). Overall, the weight of evidence suggests that thymol is not genotoxic or mutagenic.

Data Guideline 870.3550 (immune response)

The waiver rationale for immune response (870.3550) is based upon information presented in a peer-reviewed publication (Hagan et al., 1967). In the subchronic study, no effects were seen in the thymus, spleen, lymph nodes, white cell counts, red cell counts, hemoglobin counts, or hematocrits following the dosing of rats with 1000 or 10000mg/kg of food grade thymol for 19 weeks.

Note to RAL: Supplementary data illustrating thymols ability to decrease the release of prostanoids, interleukins, and leukotrienes from inflammatory cells has also been reported in human dental case studies (Skold et al., 1998; Yucel-Lindberg et al., 1999). This information will not be used in the support of this registration, but can be acknowledged scientifically.

Data Guideline 870.3700 (teratogenicity)

The waiver rationale for teratogenicity (870.3700) is based upon information presented in peer-reviewed publications. Teratogenic effects in developing chicken embryos have been reported following intra-air cell but not intra-yolk sac exposure (highest dose = 25.0 mg thymol/egg) of preincubation (day 0) or four day old chicken eggs to a solution of thymol in absolute ethanol (Verrett et al., 1980). Significant effects included phocomelia (limb shortening so that feet and wing tips arise near the body trunk), ectromelia (an absence or imperfection of one or more limbs), microphthalmia (abnormal smallness of the eye), dysgnathia (abnormality of the mouth that extends beyond teeth and includes maxilla, mandible, or both), celosomia (protrusion of the abdomen or thorax, usually accompanied by defects in sternum, ribs, and abdominal walls), and ablepharia (absence of eyelids, partial or complete). The significance of these results were not able to be verified since publication tables presented abnormalities in toto and not individually.

The relevance of these findings to data guideline requirements is questionable since there is very little in common between the development of chicken embryos and mammalian fetuses. Chick embryos have different physiology and anatomy than mammals, have different metabolic activities that operate most efficiently at different core temperatures, and are subject to different environmental stressors. For these reasons, extrapolation to mammals is problematic.

In contrast to the chicken embryo study, a report from the Environmental Risk Management Agency (ERMA, 2005) of New Zealand stated that thymol was not teratogenic in mice, rats, hamsters, and rabbits. Also, Mortazavi et al. (2003) reported no external tissue abnormalities in rat fetuses following dosing (14 days in drinking water prior to mating) of females with an infusion of the plant *Satureja khuzestanica* (which has the components thymol and carvacrol). Dose-related changes in pregnancy or the number of live offspring were also not observed in this study.

Data Guidelines 850.2100, 850.2200, 850.1075, 850.1010 (avian acute oral, avian dietary, freshwater fish LC₅₀, and freshwater invertebrate LC₅₀)

A previous memo (Carlson, 07/19/05) reported that waiver rationales presented on February 28th of 2005 for avian acute oral (850.2100), avian dietary (850.2200), freshwater fish LC₅₀ (850.1075), and freshwater invertebrate LC₅₀ (850.1010) toxicity testing in conjunction with data retrieved by EPA from ECOTOX (Carlson, 2005), and data submitted previously (MSDS dated November 15th, 2001), **supported the respective data requirements for the TGAI.**

Specifically, both avian acute and dietary, aquatic fish and invertebrate, and nontarget invertebrate waivers stipulated that no exposure was anticipated. EPA concurs with the conclusion that limited exposure will occur under the proposed conditions of use. Packaging of the gel in premeasured trays for placement directly inside the hive box mechanically precludes birds and aquatic organisms from contacting or ingesting the product. Even so, additional acceptable information on freshwater fish and invertebrate toxicity data was retrieved from EPA's EcoTox database in order to supplement this rationale. This additional information can be seen in Appendix A.

The TGAI guideline requirement for nontarget plants was not appropriate for this

submission because the product is not to be used on forests or natural grasslands, there is not indication that phytotoxicity occurs following exposure to thymol, there is no anticipated endangered or threatened species effects, and no rebuttable presumption against Special Review has been initiated.

The TGAI guideline requirement for nontarget insects was not appropriate for this submission because the product is not to be used in aquatic environments and has an unknown mechanism of action.

As with the aquatic fish and invertebrate requirements, additional data on the mortality of nontarget honeybees from thyme essential oil (39.9% thymol; limited mortality@ 8µg/bee) supplemented these ecological waivers (Albo et al., 2003). ERMA (2005) additionally reports that the safety factor between bees and mites is about 7-fold. Ellis and Baxendale (1997) reports a substantially lower safety margin (1.0-3.6 fold) when comparing bee (*Apis mellifera*) and tracheal mite (*Acarapis woodi*) toxicity. Toxicity to the tobacco cutworm has also been described (Hummelbrunner and Isman, 2001). The LD₅₀ for topical application was 25.4µg/larva and the feeding deterrent concentration DC₅₀ was 85.6 µg/cm² of leaf disk area. Synergistic acute toxicity and feeding deterrence were observed when thymol was co-incubated with trans-Anethole. Extensive field testing with thymol has revealed that thymol exposure significantly greater than that present during label application rate use of Apiguard (25 g/year) can result in increased hive agitation, absconding, or increased mortality. This precautionary statement has been included on the label. It is assumed that since Apiguard relies on contact with bees for efficacy and is to be applied directly to hives with resident bees, this product should be labeled As Toxicity Group III for bees (no bee precaution needed).

| TABLE 2. Data Requirements for TGAI (Thymol; CAS# 89-83-8; MW 150.24) | | |
|---|---|---|
| Guideline Reference No./Property | Description of Result | Methods/Source |
| Physical and Chemical Requirements | | |
| 830.6302 Color | Colorless to white | Visual inspection, MRID 46485601 |
| 830.6303 Physical State | Crystalline | Visual inspection, MRID 46485601 |
| 830.6304 Odor | Thyme-like odor | NOAA MSDS |
| 830.6313 Stability | Stable under ordinary conditions of use and storage | NOAA MSDS |
| 830.6314 Oxidation/Reduction | None of components is oxidizer or reducer | Not appropriate for this TGAI |
| 830.6315 Flammability | Not required for TGAI; Flammable | Not required for TGAI, NIH Toxnet |
| 830.6316 Explodability | Not required for TGAI | Not required for TGAI |
| 830.6317 Storage Stability | Not required for TGAI | Not required for TGAI |
| 830.6319 Miscibility | Not required for TGAI | Not required for TGAI |
| 830.6320 Corrosion Characteristics | Not required for TGAI | Not required for TGAI |
| 830.6321 Dielec Breakdown Voltage | Not required for TGAI | Not required for TGAI |
| 830.7000 pH | Neutral in alcohol | National Library of Medicine SIS ChemID Plus |
| 830.7100 Viscosity | Not required for TGAI | Not required for TGAI |
| 830.7200 Melting Range | 50.1°C, 49.5°C, 51.5°C, 48-51°C, 51°C | MRID 46485601, MSDS dated November 15 th , 2001, National Library of Medicine SIS ChemID Plus, NOAA MSDS; Barrat, 1996 |
| 830.7220 Boiling Range | Not a liquid | Not appropriate for this TGAI |
| 830.7300 Bulk Density | Specific gravity - 0.97 @ 25°C/4°C | NOAA MSDS |
| 830.7370 Dissociation Constant | 10.62 @ 20°C | National Library of Medicine SIS ChemID Plus |
| 830.7550 Partition Coefficient | Log Kow = 3.30 @ 20°C, 3.3, 3.376 | MSDS dated November 15 th , 2001, National Library of Medicine SIS ChemID Plus, Barrat, 1996 |
| 830.7840 Water Solubility | 900 mg/L water @ 20°C, 0.1g/100g water @ 25°C | National Library of Medicine SIS ChemID Plus, NOAA MSDS |
| 830.7950 Vapor Pressure | 0.0022 mmHg @ 25°C, 1 mm Hg @ 64°C, 12.7 Pa @ 40°C | Nat Lib of Medicine SIS ChemID Plus, NOAA MSDS, NIH Toxnet |
| Product Analysis Requirements | | |
| 830.1550 Product Identity | Active ingredient 99.9-100% thymol | MRID 46485601 |

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| Guideline Reference No./Property | Description of Result | Methods/Source |
|---|--|--|
| 830.1620 Manufacturing Process | | MRIDs 46664001 |
| 830.1670 Discussion of Formation of Unintentional Ingredients | No organic volatile impurities are detected following manufacture. Unintentional ingredients are not expected following manufacture. | MRIDs 46664001 |
| 830.1700 Analysis of Samples | 99.9-100% | MRID 46485601 |
| 830.1750 Certification of Limits | 100% \pm 3% = 97-100% | MRID 46485601 |
| 830.1800 Analytical Methods | Gas Liquid Chromatography | MRIDs 46485601 |
| Residue Data Requirements | | |
| 860.1500 Magnitude of the Residue | Not required for TGA1 | Not required for TGA1 |
| Toxicology Data Requirements | | |
| 870.1100 Acute Oral Toxicity | ¹ Lowest oral LD50=640 mg/kg in mice; Toxicity Category III | National Library of Medicine SIS ChemID Plus; NIOSH RTECS; Stannati et al., 1999; Aldrich Chemical Corp MSDS |
| 870.1200 Acute Dermal Toxicity | ¹ Lowest dermal 1049mg/kg in mice using DAF = 0.61; Toxicity Category II | EPA Thymol RED, ERMA 2005 |
| 870.1300 Acute Inhalation Toxicity | Inhalation LD50 > 5mg/L; Toxicity Category IV | FDA CDER inactive component @ 0.01% in halothane anesthetics (5%) |
| 870.2400 Primary Eye Irritation | Corrosive; Toxicity Category I | cited in NIOSH RTECS |
| 870.2500 Primary Dermal Irritation | Corrosive; Toxicity Category I | cited in NIOSH RTECS; Barrat, 1996 |
| 870.2600 Hypersensitivity | Sensitizer - Thymol | Hostynek and Magee, 1997 |
| Hypersensitivity Incidents | Not required for TGA1 | Not required for TGA1 |
| 870.5000 Genotoxicity | ¹ Negative for Mutagenesis/Genotoxicity based on weight of evidence | Azizan and Blevins, 1995; Zani et al., 1990; Stannati et al., 1999; Evrim et al., 2003; ERMA, 2005. |
| 870.3550 Immune Response | No Subchronic Immune Effects | Hagan et al., 1967 |
| 870.3100 90-day Feeding (1 spp) | No Anticipated Subchronic Occupational Exposure; Dietary Exposure Insignificant | Waiver Rationale |
| 870.3250 90-day Dermal (1 spp) | No Anticipated Subchronic Occupational Exposure; Dietary Exposure Insignificant | Waiver Rationale |

| Guideline Reference No./Property | Description of Result | Methods/Source |
|--|--|--|
| 870.3465 90-day Inhalation (1 spp) | No Anticipated Subchronic Occupational Exposure; Dietary Exposure Insignificant | Waiver Rationale |
| 870.3700 Teratogenicity (1 spp) | Not teratogenic effects in mice, rats, hamsters, and rabbits | ERMA, 2005 |
| Nontarget Organism, Fate, and Expression Requirements | | |
| 850.2100 Avian Acute Oral | No Anticipated Exposure | Feb 28, 2005 admin materials waiver rationale |
| 850.2200 Avian Dietary | No Anticipated Exposure | Feb 28, 2005 admin materials waiver rationale |
| 850.1075 Freshwater Fish LC50 | No Anticipated Exposure, <i>P. promelas</i> , 96h LC50 - 3.2mg/L; other | Feb 28, 2005 admin materials waiver rationale, ECOTOX database |
| 850.1010 Freshwater Invertebrate LC50 | No Anticipated Exposure, <i>D. magna</i> 96h LC50 - 1.7mg/L; other | Feb 28, 2005 admin materials waiver rationale, ECOTOX database |
| Nontarget Plant Studies | No phytotoxicity issues, no anticipated endangered or threatened species effects, no rebuttable presumption against Special Review initiated | Not appropriate for this TGAI |
| Nontarget Insect Studies | Not introduced into aquatic environment, not introduced into uncontrolled terrestrial environment. Some mortality at 8 µg/honeybee for thyme essential oil; Toxicity Group III | Not appropriate for this TGAI, Albo et al., 2003 |

¹ see the accompanying text

Appendix A. Aquatic Fish and Invertebrate Toxicity Data from EPA's EcoTox Database

| Test Loc | CAS # | Chemical Name | Scientific Name | Common Name | Endpoint | Effect | Effect Measurement | Media Type | Test Duration (hours) | Exposure Type | Citation | Concentration Mean (ug/L) |
|----------|-------|---------------|--------------------------|--------------------------------|----------|--------|--------------------|------------|-----------------------|---------------|----------|---------------------------|
| LAB | 89838 | Thymol | Aesilus intermedius | Aquatic sowbug | LC50 | MOR | MORT | FW | 96 | S | 1 | 17000 |
| LAB | 89838 | Thymol | Aesilus intermedius | Aquatic sowbug | LC50 | MOR | MORT | FW | 96 | S | 1 | 25000 |
| LAB | 89838 | Thymol | Aesilus intermedius | Aquatic sowbug | LC50 | MOR | MORT | FW | 96 | S | 1 | 28000 |
| LAB | 89838 | Thymol | Aesilus intermedius | Aquatic sowbug | LC50 | MOR | MORT | FW | 96 | S | 1 | 24000 |
| LAB | 89838 | Thymol | Aesilus intermedius | Aquatic sowbug | LC50 | MOR | MORT | FW | 96 | S | 1 | 26000 |
| LAB | 89838 | Thymol | Aesilus intermedius | Aquatic sowbug | LC50 | MOR | MORT | FW | 96 | S | 1 | 19000 |
| LAB | 89838 | Thymol | Daphnia magna | Water flea | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Daphnia magna | Water flea | LC50 | MOR | MORT | FW | 96 | S | 1 | 1700 |
| LAB | 89838 | Thymol | Daphnia magna | Water flea | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Daphnia magna | Water flea | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Daphnia magna | Water flea | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Daphnia magna | Water flea | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Dugesia tigrina | Turbellarian, flatworm | LC50 | MOR | MORT | FW | 96 | S | 1 | 5900 |
| LAB | 89838 | Thymol | Dugesia tigrina | Turbellarian, flatworm | LC50 | MOR | MORT | FW | 96 | S | 1 | 4000 |
| LAB | 89838 | Thymol | Dugesia tigrina | Turbellarian, flatworm | LC50 | MOR | MORT | FW | 96 | S | 1 | 3600 |
| LAB | 89838 | Thymol | Dugesia tigrina | Turbellarian, flatworm | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Dugesia tigrina | Turbellarian, flatworm | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Dugesia tigrina | Turbellarian, flatworm | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Dugesia tigrina | Turbellarian, flatworm | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Gammarus fasciatus | Scud | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Gammarus fasciatus | Scud | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Gammarus fasciatus | Scud | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Gammarus fasciatus | Scud | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Gammarus fasciatus | Scud | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Gammarus fasciatus | Scud | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Helisoma trivolvis | Ramshorn snail | LC50 | MOR | MORT | FW | 96 | S | 1 | 32000 |
| LAB | 89838 | Thymol | Helisoma trivolvis | Ramshorn snail | LC50 | MOR | MORT | FW | 96 | S | 1 | 32000 |
| LAB | 89838 | Thymol | Helisoma trivolvis | Ramshorn snail | LC50 | MOR | MORT | FW | 96 | S | 1 | 32000 |
| LAB | 89838 | Thymol | Helisoma trivolvis | Ramshorn snail | LC50 | MOR | MORT | FW | 96 | S | 1 | 32000 |
| LAB | 89838 | Thymol | Helisoma trivolvis | Ramshorn snail | LC50 | MOR | MORT | FW | 96 | S | 1 | 32000 |
| LAB | 89838 | Thymol | Helisoma trivolvis | Ramshorn snail | LC50 | MOR | MORT | FW | 96 | S | 1 | 32000 |
| LAB | 89838 | Thymol | Lumbriculus variegatus | Oligochaete, worm | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Lumbriculus variegatus | Oligochaete, worm | LC50 | MOR | MORT | FW | 96 | S | 1 | 4800 |
| LAB | 89838 | Thymol | Lumbriculus variegatus | Oligochaete, worm | LC50 | MOR | MORT | FW | 96 | S | 1 | 10000 |
| LAB | 89838 | Thymol | Lumbriculus variegatus | Oligochaete, worm | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Lumbriculus variegatus | Oligochaete, worm | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Lumbriculus variegatus | Oligochaete, worm | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Pimephales promelas | Fathead minnow | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Pimephales promelas | Fathead minnow | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Pimephales promelas | Fathead minnow | LC50 | MOR | MORT | FW | 96 | S | 1 | 4200 |
| LAB | 89838 | Thymol | Pimephales promelas | Fathead minnow | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Pimephales promelas | Fathead minnow | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Pimephales promelas | Fathead minnow | LC50 | MOR | MORT | FW | 96 | S | 1 | 3200 |
| LAB | 89838 | Thymol | Lymnaeidae | Pond snail family | NR | MOR | MORT | FW | 24 | NR | 2 | 10000 |
| LAB | 89838 | Thymol | Oncorhynchus kisutch | Coho salmon, silver salmon | NR | BEH | EQUIL | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Oncorhynchus kisutch | Coho salmon, silver salmon | NR | BEH | EQUIL | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Oncorhynchus kisutch | Coho salmon, silver salmon | NR | MOR | MORT | FW | 24 | S | 3 | 10000 |
| LAB | 89838 | Thymol | Oncorhynchus kisutch | Coho salmon, silver salmon | NR | MOR | MORT | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Oncorhynchus kisutch | Coho salmon, silver salmon | NR | MOR | MORT | FW | 24 | S | 3 | 10000 |
| LAB | 89838 | Thymol | Oncorhynchus kisutch | Coho salmon, silver salmon | NR | MOR | MORT | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Oncorhynchus kisutch | Coho salmon, silver salmon | NR | MOR | MORT | FW | 24 | S | 3 | 10000 |
| LAB | 89838 | Thymol | Oncorhynchus mykiss | Rainbow trout, doreidson trout | NR | BEH | EQUIL | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Oncorhynchus mykiss | Rainbow trout, doreidson trout | NR | BEH | EQUIL | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Oncorhynchus mykiss | Rainbow trout, doreidson trout | NR | MOR | MORT | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Oncorhynchus mykiss | Rainbow trout, doreidson trout | NR | MOR | MORT | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Oncorhynchus tshawytscha | Chinook salmon | NR | BEH | EQUIL | FW | 24 | S | 3 | 10000 |
| LAB | 89838 | Thymol | Oncorhynchus tshawytscha | Chinook salmon | NR | BEH | EQUIL | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Oncorhynchus tshawytscha | Chinook salmon | NR | MOR | MORT | FW | 24 | S | 3 | 10000 |
| LAB | 89838 | Thymol | Oncorhynchus tshawytscha | Chinook salmon | NR | MOR | MORT | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Oncorhynchus tshawytscha | Chinook salmon | NR | MOR | MORT | FW | 24 | S | 3 | 10000 |
| LAB | 89838 | Thymol | Pychocheilus oregonensis | Northern squawfish | NR | BEH | EQUIL | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Pychocheilus oregonensis | Northern squawfish | NR | BEH | EQUIL | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Pychocheilus oregonensis | Northern squawfish | NR | BEH | EQUIL | FW | 24 | S | 3 | 10000 |
| LAB | 89838 | Thymol | Pychocheilus oregonensis | Northern squawfish | NR | BEH | EQUIL | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Pychocheilus oregonensis | Northern squawfish | NR | MOR | MORT | FW | 24 | S | 3 | 10000 |
| LAB | 89838 | Thymol | Pychocheilus oregonensis | Northern squawfish | NR | MOR | MORT | FW | 24 | S | 3 | 2000 |
| LAB | 89838 | Thymol | Pychocheilus oregonensis | Northern squawfish | NR | MOR | MORT | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Pychocheilus oregonensis | Northern squawfish | NR | MOR | MORT | FW | 24 | S | 3 | 5000 |
| LAB | 89838 | Thymol | Pychocheilus oregonensis | Northern squawfish | NR | MOR | MORT | FW | 24 | S | 3 | 10000 |
| LAB | 89838 | Thymol | Pychocheilus oregonensis | Northern squawfish | NR | MOR | MORT | FW | 24 | S | 3 | 10000 |
| LAB | 89838 | Thymol | Pychocheilus oregonensis | Northern squawfish | NR | MOR | MORT | FW | 24 | S | 3 | 10000 |

Note To RAL: Additional toxicology studies using non-traditional routes of exposure or expression have been reported for thymol and its closely related chemical analogues. Additional toxicological evidence include; a thymol oral LD_{low} (cat - 100 mg/kg; rabbit - 100mg/kg), subcutaneous LD_{low} (rabbit - 1000 mg/kg; frog - 75 mg/kg), subcutaneous LD_{50} (mice - 680 mg/kg), intravenous LD_{50} (mice - 80 mg/kg; dog - 310 mg/kg) and intraperitoneal LD_{50} (mice - 73.3 mg/kg). Clinical signs such as somnolence, ataxia, convulsions, sleep, and dyspnea were reported following dosing.

One publication reported the intravenous LD_{50} of o-thymol (phenol, 2-isopropyl-6-methyl-; CAS# 3228044). Following dosing of the mice, behavioral changes were observed (sleep) and an LD_{50} was calculated (80 mg/kg; JMCMAR, 1980).

Another publication also reported additional toxicological evidence on the subcutaneous LD_{50} (184 mg/kg) and behavioral changes (somnolence) following dosing (OSDIAF, 1956).

Note To RAL: In vitro studies have identified additional target tissues that thymol may affect. These include the nervous system, skeletal and cardiac muscle, and the skeletal system. Haeseler et al. (2002) reported that thymol blocked voltage-operated sodium channels in rat neuronal and human skeletal muscle with a potency similar to the local anesthetic lidocaine. Priestley et al. (2003) expanded this argument by reporting that thymol potentiates GABA_A receptor activity by binding at an unidentified receptor site (not benzodiazepine/ β -carboline, steroid, or loreclezole receptor binding sites). Magyar et al. (2002) also reported that thymol suppresses cardiac potassium and calcium ionic channels and alters the function of ventricular cardiomyocytes. Similar results were reported by Szentandrassy et al. (2003) in rat skeletal muscle fibers. Finally, Muhlbauer et al. (2003) reported that thymol and essential oils extracted from thyme directly inhibit bone resorption in the rat.

In vitro reports may explain in part why thymol has antinociceptive and local anesthetic properties. Blockage of neural sodium channels is the primary mechanism of action for most local anesthetics. Activity at the GABA_A receptor may also explain insecticidal and molluscicidal properties and suggest potential adverse effects in mammals. Positive allosteric modulators of GABA_A in insects (abamectin) can affect feeding and egg laying. In humans, modulation of GABA_A can induce behavioral effects such as anxiolysis, mitigation of convulsions, sedation, and anesthesia.

Overall, neural, muscular, and bone effects are of limited importance considering the current use of thymol (acaricides in honeybee hives). Should pesticidal uses for thymol expand and increased exposure become an issue, further investigation into these potential effects may be prudent.

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Information for the Exemption from the Requirement of a Tolerance

PRODUCT NAME and PROPOSED USE:

Vita (Europe) Limited in c/o Landis International, Inc. has submitted a petition for exemption from the requirements of a tolerance for the use of thymol in bee hives. The food commodities affected by the proposed use are honey and beeswax. Supporting documents have been provided by the registrant and EPA. Thymol is the active ingredient in Apiguard, an acaricide. The proposed application of Apiguard to the inside of bee hives is made to decrease the incidence of Varroa mite infestation.

The proposed hive (honey and beeswax) use for Apiguard stipulates that it is to be applied up to twice per year (100g Apiguard; 25.0 grams thymol/year) to bee hives boxes when Varroa mite infestation is suspected. Waterproof gloves, eye protection, long pants, a long sleeved shirt, shoes and socks are required to apply the packages of Apiguard. Applications should not occur during honey flow or when the temperature is $<60^{\circ}\text{F}$ or $>105^{\circ}\text{F}$.

At hives to be treated, the supers have to be removed prior to Apiguard application. The foil lid on the Apiguard aluminum tray (one at a time per hive box) is peeled back leaving one corner attached. The open tray is set inside the hive on the top of the brood frames, open side up. The hive box is closed, leaving approximately one-quarter inch of free space in between the top of the tray and the hive cover board. Monitor the mite fall. After two weeks, 80-90% of the gel should be gone from the tray. The second tray of Apiguard should be applied after two weeks (remove the first tray) if needed and left until empty. The trays along with any residual Apiguard should be removed prior to re-installing the supers.

A. PRODUCT CHEMISTRY and MANUFACTURING PROCESS:

Thymol is a colorless to white crystalline solid, that smells like thyme, is stable under ordinary conditions of use and storage, has a neutral pH in alcohol, is not an oxidizer or reducer, is not corrosive, melts at $48 - 51.5^{\circ}\text{C}$, boils at $232.5-233^{\circ}\text{C}$, has a specific gravity of 0.97 @ $25^{\circ}\text{C}/4^{\circ}\text{C}$, a dissociation constant of 10.62 @ 20°C , a partition coefficient of 3.3-3.34 (Log Kow), water solubility of 900mg/L @ 20°C or 0.1g/100g water @ 25°C , and vapor pressure of 0.0022 mm Hg @ 25°C , 12.7 Pa @ 40°C , and 1mm Hg @ 64°C .

The thymol used in Apiguard is created synthetically, not extracted from plants. In brief, the starting materials [REDACTED]

[REDACTED]

[REDACTED]

All data requirements concerning product chemistry and manufacturing process for both Apiguard and the TGAi thymol have been provided by the registrant and supplemented by EPA. These submissions were acceptable.

B. PRODUCT IDENTITY/CHEMISTRY:

1) Nature of the Pesticide and Residue Identity:

Five years of European field trials have demonstrated that thymol residues are relatively stable under standard environmental conditions (1992-1996; Imdorf, 2003.). Fall application of thymol to infested hives resulted in detectable thymol residues in the following spring's honey at 3 sites (ranging from 0.05-0.5, 0.12-0.31, and 0.0-0.07 mg/kg). The provided information is acceptable.

2) Magnitude of the Residue:

Field residue studies assessing thymol incorporation into honey and beeswax were performed in Maryland, California, and Texas (MRID 46254301) and Europe (1997-1998, MRID 46043510) and submitted by the registrant.

In the U.S. trials, four treated and four untreated control hives were used per site (MD, CA, or TX). Each treated hive received 2 trays total of Apiguard (25 g thymol total) with a 2 week interval between trays. After the second set of thymol trays was removed (28-30 days following placement), new supers for building honeycomb and collecting honey were placed in each hive. Hive honey and beeswax were collected 30 days following this. New frames were then put in the supers for the 60 day collection. All samples were frozen (≤ 35 days) before extraction and analyzed using gas-chromatography with flame ionization (honey LOQ = 0.01 mg/kg, beeswax LOQ = 0.10 mg/kg). Thymol residues in honey ranged from 0.019 - 2.59 mg/kg by day 31 and $< \text{LOQ}$ - 0.96 mg/kg by day 61. Thymol residues in beeswax ranged from 0.11 - 97.6 mg/kg by day 31 and $< \text{LOQ}$ - 18.7 mg/kg by day 61. No thymol residues were detected in untreated hives.

In the European trials, 2-10 samples were gathered from treated and control hives per study. In four studies, each treated hive received 2 trays total of Apiguard (25 g thymol total) in various hive locations (on top of brood frames, on bottom of brood frames) with a 15 day interval between trays. In one other study, each treated hive received 3 trays total of Apiguard (37.5 g thymol total) with each tray replaced at 10 day intervals. Following treatment end, the supers were put into the hive boxes. In one trial, samples were collected from the brood frame on the last day of treatment and the super 30 days after treatment end. In two of the studies samples were collected from the brood frame 2 days after treatment end and the supers 103 days after treatment. In the last two studies, treatment occurred during honeyflow and samples were collected from the brood nest 2 and 14 days after treatments end. All samples were frozen (≤ 35 days) before extraction and analyzed using gas-chromatography with flame ionization (honey and wax LOQ = 0.03 mg/kg). Thymol residues in honey ranged from $< \text{LOQ}$ - 4.61 mg/kg at day 2 and 0.86 - 1.48 mg/kg at day 103. Thymol residues in beeswax ranged from 1.83 - 51.56 mg/kg at day 2 and 1.18 - 38.43 mg/kg by day 14. No thymol residues were detected in untreated hives. The provided information is acceptable.

3) Analytical Method:

An analytical method for measuring thymol in honey and beeswax was submitted by the registrant (MRIDs 46043511, -12, -13). In brief, beeswax and honey samples were spiked with thymol (98% purity) and mixed with distilled water and hydrochloric acid. The mixture was then

placed on a heating mantle connected to distillation apparatus. The mixture was heated for 1-2 hours and distillate collected. The distillate was adjusted to $\text{pH} > 10$ with sodium hydroxide, transferred to a separation funnel, and partitioned with hexane. The aqueous phase containing thymol was drained into a separate flask, and partitioned with dichloromethane. After phase separation, the lower phase containing dichloromethane and thymol was drained through a funnel containing 20-30 grams of sodium sulfate. The dichloromethane was evaporated from this filtrate residue. Hexane was added to the sample residue and the mix transferred to Bakerbond extraction cartridges connected to a solid-phase extraction manifold. Thymol was eluted from the cartridge with hexane/dichloromethane, rinsed with dichloromethane, and evaporated once more. This final residue was diluted with hexane/acetone and analyzed using gas chromatography with flame ionization (HP 5890 Ser. II with a 30m x 0.25 mm DB 1701 column and a HP MS5971A Chemstation detector). Duplicate samples were quantified by peak height standardized to a reference calibration curve. The method limit of quantitation (LOQ) ranged from 0.03 - 3.0 mg/kg in honey to 0.03 - 30 mg/kg in beeswax with % recoveries of 82-94% ($\pm 12-19\%$). Higher LOQ's were thought to have resulted from matrix interference. The provided information is acceptable.

C. MAMMALIAN TOXICOLOGY DATABASE:

No studies illustrating thymol (TGAI) induced mammalian toxicity were submitted by the registrant. In lieu of these studies the registrant submitted waiver justifications. These waiver justifications were supplemented by EPA. There are no data gaps to address for thymol.

1) Acute and Short-term Toxicity:

Thymol was placed into Toxicity Category IV for acute inhalation toxicity, Toxicity Category III for acute oral toxicity, and Toxicity Category II for acute dermal toxicity. Thymol was placed into Toxicity Category I for primary eye irritation and primary skin irritation, and conservatively, was a dermal sensitizer. Apiguard was placed into Toxicity Category IV for primary dermal irritation, Toxicity Category III for acute oral and acute dermal toxicity, Toxicity Category I for primary eye irritation, and was not a dermal sensitizer. A Toxicity Category of III was conservatively given to Apiguard for acute inhalation toxicity, even though a suitable aerosol atmosphere was unable to be generated for the pesticide.

No thymol-induced short term alterations in rat weight, food intake, general condition, hematology (white blood cells, red blood cell counts, hemoglobin, and hematocrit), organ weight (liver, kidneys, spleen, heart, and testes), abdominal and thoracic viscera, bone, bone marrow, and muscle were found in a study investigating the oral toxicity of dietary thymol exposure (doses 1,000 and 10,000 mg/kg).

2) Genotoxicity:

Genotoxicity and mutagenicity studies were submitted as waiver rationales for genotoxicity (870.5000). These, in combination with other peer-reviewed publications retrieved by EPA, **support the respective data requirement for the TGAI.**

Thymol has been reported to be non-mutagenic in multiple Ames tests (strains TA97,

TA98, and TA100 w and w/out metabolic transformation with S9 incubation (Azizan and Blevins, 1995; MRID 46282801; Stamatii et al., 1999), but positive in unscheduled DNA synthesis (liquid scintillation), sister chromatid exchange, and cell transformation tests in Syrian hamster embryo cells in culture (Zani et al., 1991, MRID 46282802; ERMA, 2005; Tsutsui, 1987). In addition, thymol does not induce chromosomal aberrations in *Allium cepa* (Grant, 1982)

The chemical isomer of thymol, carvacrol, does not form sister chromatid exchanges in *in vitro* assays with human peripheral blood lymphocytes and inhibits SCE induction by mitomycin C (Ipek et al., 2003).

Steam distilled extracts of three species of *Thymus* (*capitatus*, *citriodorus*, *vulgaris*) also were negative for DNA damaging activity and mutagenicity in the Ames test (strains TA1535, TA1537, TA98, and TA100 with and w/out metabolic activation). They were also non-mutagenic in a salmonella/microsome assay, did not induce the formation of micronuclei in mice, even when orally dosed in the toxic range (1100 mg/kg bw). Further, in the A/He strain of mice, thymol did not increase the incidence of spontaneous lung tumors following repeated intraperitoneal dosing (Anonymous, 2000). Overall, the weight of evidence suggests that thymol is not genotoxic or mutagenic.

3) Reproductive and Developmental Toxicity:

Teratogenic effects in developing chicken embryos have been reported following intra-air cell but not intra-yolk sac exposure (highest dose = 25.0 mg thymol/egg) of preincubation (day 0) or four day old chicken eggs to a solution of thymol in absolute ethanol (Verrett et al., 1980). Significant effects included phocomelia (limb shortening so that feet and wing tips arise near the body trunk), ectromelia (an absence or imperfection of one or more limbs), microphthalmia (abnormal smallness of the eye), dysgnathia (abnormality of the mouth that extends beyond teeth and includes maxilla, mandible, or both), celosomia (protrusion of the abdomen or thorax, usually accompanied by defects in sternum, ribs, and abdominal walls), and ablepharia (absence of eyelids, partial or complete). The significance of these results were not able to be verified since publication tables presented abnormalities in toto and not individually.

The relevance of these findings to data guideline requirements is questionable since there is very little in common between the development of chicken embryos and mammalian fetuses. Chick embryos have different physiology and anatomy than mammals, have different metabolic activities that operate most efficiently at different core temperatures, and are subject to different environmental stressors. For these reasons, extrapolation to mammals is problematic.

In contrast to the chicken embryo study, a report from the Environmental Risk Management Agency (ERMA, 2005) of New Zealand states that thymol was not teratogenic in mice, rats, hamsters, and rabbits. Also, Mortazavi et al. (2003) reported no external tissue abnormalities in rat fetuses following dosing (14 days in drinking water prior to mating) of females with an infusion of the plant *Satureja khuzestanica* (which has the components thymol and carvacrol). Dose-related changes in pregnancy or the number of live offspring were also not observed in this study.

4) Subchronic Toxicity:

Oral subchronic studies are typically required when the pesticidal use requires a tolerance or an exemption from the requirement of a tolerance, a food additive regulation, or its use results in repeated human oral exposure. The subacute and chronic study submitted as a waiver rationale for the subchronic toxicity (870.3100), and other rationales presented by EPA, **support the respective data requirement for the TGA1**. Sole support was not justified for the submitted study because certain critical aspects of the subchronic study (dose levels, number of animals, toxicity endpoints) were not reported or performed.

Although the use of thymol requires a tolerance or exemption from the requirement of a tolerance, its use as a food additive has already been established. Thymol is currently approved by the U.S. Food and Drug Administration (FDA) as a synthetic flavoring substance for the direct addition to food for human consumption (FDA, 21CFR §172.515) and as a preservative and indirect food additive of adhesives (FDA, 21CFR §175.105). The source plant of thymol, thyme or wild and creeping thyme (*Thymus vulgaris* L., or *Thymus serpyllum* L.) is also currently acknowledged by FDA as a spice, natural oil, oleoresin, or natural extract that is generally recognized as safe (21CFR §182.10, 21CFR §182.20). Although constituents vary depending on species and cultivation method, thyme can be comprised of up to 60% thymol (De Vincenzi et al., 1991).

The use pattern for Apiguard (25% thymol) precludes subchronic (daily, intermediate-term) occupational exposure. Only two applications of Apiguard, separated by 2 weeks, are made per hive. Applications are synchronized within apiaries to prohibit repopulation of untreated or previously treated hives with Varroa mites. In addition, placement within hives further mitigates inhalation and dermal exposure. Therefore, it is anticipated that occupational exposures will be of acute duration only and not subchronic.

Dietary subchronic exposure to thymol in honey is probable. Thymol residues occur naturally in some forms of lime honey (0.02-0.16 mg/kg) and thymol residues in honey (maximum 0.5 mg/kg) have been reported to be present 6 months following thymol treatment in European field studies spanning 5 years.

Thymol residues are found in other food stuffs at significantly higher concentrations than those resulting from pesticidal treatments. Thymol has been found in ice cream (44 mg/kg), non-alcoholic beverages (2.5-11 mg/kg), candy (9.4 mg/kg), baked goods (5.0-6.5 mg/kg), and chewing gum (100 mg/kg). It is also a constituent in cooking herbs derived from wild or creeping thyme (up to 60%) and mandarin and tangerine oils (0.1-0.03%). Because the dietary contribution of thymol from honey is expected to be negligible compared to that already in the diet, subchronic studies are not required.

This waiver rationale for 90-day feeding (870.3100), 90-day dermal toxicity (870.3250), and 90-day inhalation toxicity (870.3465) studies, therefore, supports the respective data requirements for the TGA1.

5) Chronic Toxicity/Cancer:

Chronic toxicity/cancer studies are required when the potential for adverse chronic effects are indicated (by subchronic study results, the pesticide use pattern, or potential for exposure) or the active ingredients or their metabolites (etc) produces a morphologic effect that could lead to neoplasia (hyperplasia, metaplasia) or if adverse cellular effects suggest oncogenic potential.

The subacute and chronic study submitted as a waiver rationale for chronic toxicity/ oncogenicity (870.4100, 870.4200), and other rationales presented by EPA **support the respective data requirement for the TGAI**. Sole support was not justified for the submitted study because certain critical aspects of the chronic study (study duration, dose levels, number of animals, toxicity endpoints) were not reported or performed.

Genotoxicity and mutagenicity studies suggest via a weight of negative evidence, that thymol does not have cellular oncogenic potential. Further, no lesion indicative or predictive of neoplasia has been determined in reviewed studies.

Thymol is currently approved by the U.S. Food and Drug Administration (FDA) as a synthetic flavoring substance for the direct addition to food for human consumption (FDA, 21CFR §172.515) and as a preservative and indirect food additive of adhesives (FDA, 21CFR §175.105). The source plant of thymol, thyme or wild and creeping thyme (*Thymus vulgaris* L., or *Thymus serpyllum* L.) is also currently acknowledged by FDA as a spice, natural oil, oleoresin, or natural extract that is generally recognized as safe (21CFR §182.10, 21CFR §182.20). Although constituents vary depending on species and cultivation method, thyme can be comprised of up to 60% thymol (De Vincenzi et al., 1991).

The use pattern for Apiguard (25% thymol) precludes chronic (daily, long-term) occupational exposure. Only two applications of Apiguard, separated by 2 weeks, are made per hive. Applications are synchronized within apiaries to prohibit repopulation of untreated or previously treated hives with Varroa mites. In addition, placement within hives further mitigates inhalation and dermal exposure. Therefore, it is anticipated that occupational exposures will be of acute duration only and not chronic.

Dietary chronic exposure to thymol in honey is probable. Thymol residues occur naturally in some forms of lime honey (0.02-0.16 mg/kg) and thymol residues in honey (maximum 0.5 mg/kg) have been reported to be present 6 months following thymol treatment in European field studies spanning 5 years.

Thymol residues are also found in other food stuffs at significantly higher concentrations than those resulting from Apiguard treatments. Thymol has been found in ice cream (44 mg/kg), non-alcoholic beverages (2.5-11 mg/kg), candy (9.4 mg/kg), baked goods (5.0-6.5 mg/kg), and chewing gum (100 mg/kg). It is also a constituent in cooking herbs derived from wild or creeping thyme (up to 60%) and mandarin and tangerine oils (0.1-0.03%). Because the dietary contribution of thymol from honey is expected to be negligible compared to that already in the diet, chronic studies are not required.

This waiver rationale for chronic toxicity/oncogenicity (870.4100, 870.4200) studies, therefore, support the respective data requirements for the TGAI.

6) Metabolic Studies:

Supplementary metabolic studies have been reviewed by EPA. Metabolic studies in rats revealed that most of the thymol was excreted unchanged or as a glucuronide or sulfate conjugate in the urine within 24 hours (Austgulen et al., 1987). A limited amount of thymol was also metabolized into 2-(2-hydroxy-4-methylphenyl)propan-1-ol > 5-hydroxymethyl-2-(1-methylethyl)phenol = 2-(2-hydroxy-4-methylphenyl)propionic acid > 2,5-dihydroxy-p-cymene = 3-hydroxy-4-(1-methylethyl)benzoic acid > 2-(4-hydroxymethyl-2-hydroxyphenyl)propan-1-ol and excreted in the urine. The predominate metabolic reaction was oxidation of the methyl and

isopropyl groups and not ring hydroxylation. This was true also for carvacrol, which had similar metabolic degradates. In rabbits dosed with thymol, thymol glucuronide, an acetyl derivative of methyl glucuronate was identified in the urine (Takada, et al., 1979)

In a similar study, 18 urinary metabolites were collected and identified following the dosing of p-cymene to rats and guinea pigs. As with thymol and carvacrol, oxidation of the methyl and isopropyl group occurred extensively. No ring hydroxylation was detected in rats, but in guinea pigs, carvacrol and hydroxycarvacrol were formed in minute amounts (Walde et al., 1983).

D. AGGREGATE EXPOSURE:

1) Dietary Exposure:

No thymol dietary exposure studies were submitted by the registrant. A deterministic dietary assessment was performed by EPA comparing current per capita consumption of thymol in ice cream, carbonated cola beverages, yellow cake with white icing, and caramel candies to consumption of maximal thymol in honey residues (4.61 mg/kg) reported from European field studies.

Food:

Thymol is found naturally in some forms of lime honey (0.02-0.16 mg/kg), in cooking herbs derived from wild or creeping thyme (up to 60%), bilberry (0.001 mg/kg), cranberry (trace), and mandarin and tangerine oils (0.1-0.03%). Thymol is also present (added to) in other food stuffs such as ice cream (44 mg/kg), non-alcoholic beverages (2.5-11 mg/kg), candy (9.4 mg/kg), baked goods (5.0-6.5 mg/kg), and chewing gum (100 mg/kg).

Thymol is currently approved by the U.S. Food and Drug Administration (FDA) as a synthetic flavoring substance for the direct addition to food for human consumption (FDA, 21CFR §172.515) and as a preservative and indirect food additive of adhesives (FDA, 21CFR §175.105). The source plant of thymol, thyme or wild and creeping thyme (*Thymus vulgaris* L., or *Thymus serpyllum* L.) is also currently acknowledged by FDA as a spice, natural oil, oleoresin, or natural extract that is generally recognized as safe (21CFR §182.10, 21CFR §182.20).

European studies using Apiguard in bee hives *during honey flow* demonstrated that thymol residues in honey accumulated up to 4.61 mg thymol/kg honey after 2 days of exposure. This represents a worst case scenario for potential residues because residue incorporation into honey could have occurred directly from the Apiguard tray. Thymol residues in wax were not considered in this dietary assessment because wax is not known to be a dietary foodstuff.

EPA estimated the dietary exposure to U.S. subpopulations using the maximal thymol residue level from the European studies (4.61 mg/kg) and compared it to estimated exposures resulting from thymol in other foodstuffs (ice cream @ 44 mg/kg, yellow cake @ 6.5 mg/kg, cola beverage 2 11 mg/kg, and caramel candy @ 9.4 mg/kg). Ingestion rates for honey and the foodstuffs were obtained from the FDA Total Diet Study (1990). Body weights for the respective populations were derived from the EPA Exposure Factors Handbook (1997).

Dietary Exposure Results and Characterization

Calculated thymol exposures from honey were substantially less than that from the food stuffs (Table 1). Normalized data showed that the U.S. general population is potentially exposed to 938 times more thymol from the consumption of ice cream, yellow cake, cola beverages, and caramel candy than from thymol consumed in honey (Table 2). Similarly, calculations show that the population with highest exposure (6 year old child) is potentially exposed to 279 times more thymol from the consumption of other foodstuffs than from thymol in honey. Male adults (60-65 years old) share a similar level of exposure with 251 times more exposure to thymol from foodstuffs other than honey. These calculations illustrate that thymol residues in honey will not contribute significantly to the dietary burden of thymol.

The dietary risks of thymol in honey, therefore, are **below EPA's level of concern** for all population subgroups. The conservative nature of this dietary assessment ensured that the risks from exposure to thymol in honey were not underestimated.

Table 1. Summary of Dietary Exposure from Thymol residues in honey

| U.S. Subpopulation | ¹ Body Weight (kg) | ² Honey Ingested per Day (mg) | Residue Exposures (mg/kg/day) | | | | |
|----------------------------|-------------------------------|--|---------------------------------|-----------------------------------|--------------------------------------|---------------------------------------|--|
| | | | ³ Honey (4.61 mg/kg) | ⁴ Ice Cream (44 mg/kg) | ⁴ Yellow Cake (6.5 mg/kg) | ⁴ Cola Beverage (11 mg/kg) | ⁴ Caramel Candy (9.4 mg/kg) |
| General U.S. | 72 | 419.42 | 0.000027 | 0.00829 | 0.00038 | 0.01656 | 0.00003 |
| Infant (6-11 mo) | 9 | 0.00 | 0.000000 | 0.00188 | 0.0003 | 0.00162 | 0.00000 |
| Child (2 yr) | 13 | 133.76 | 0.000046 | 0.01936 | 0.00091 | 0.0346 | 0.00034 |
| Child (6 yr) | 23 | 1136.94 | 0.000232 | 0.03681 | 0.00152 | 0.02632 | 0.000065 |
| Child (10 yr) | 36 | 278.41 | 0.000035 | 0.02965 | 0.0003 | 0.02285 | 0.000033 |
| Female Juvenile (14-16 yr) | 57 | 272.93 | 0.000022 | 0.01475 | 0.0005 | 0.03378 | 0.000066 |
| Male Juvenile (14-16 yr) | 62 | 209.05 | 0.000016 | 0.01511 | 0.00047 | 0.04416 | 0.000014 |
| Female Adult (25-30 yr) | 64 | 217.11 | 0.000016 | 0.00847 | 0.00019 | 0.02705 | 0.000014 |
| Male Adult (25-30 yr) | 79 | 312.49 | 0.000018 | 0.00666 | 0.00022 | 0.02974 | 0.000025 |
| Female Adult (40-45 yr) | 67 | 340.13 | 0.000023 | 0.00633 | 0.00042 | 0.01247 | 0.000069 |
| Male Adult (40-45 yr) | 81 | 158.19 | 0.000009 | 0.00783 | 0.0006 | 0.01833 | 0.00019 |
| Female Adult (60-65 yr) | 68 | 218.55 | 0.000015 | 0.00659 | 0.00052 | 0.00674 | 0.00000 |
| Male Adult (60-65 yr) | 79 | 1146.21 | 0.000067 | 0.00826 | 0.00045 | 0.0081 | 0.000022 |
| Female Adult (70 yr) | 67 | 701.83 | 0.000049 | 0.00869 | 0.00048 | 0.00479 | 0.000013 |
| Male Adult (70 yr) | 75 | 863.20 | 0.000053 | 0.01104 | 0.00058 | 0.00721 | 0.000009 |

¹ Taken from EPA Exposure Factors Handbook (1997)

² From FDA Total Diet Study (1990)

³ From registrant-submitted European field residue study (MRID 46043510)

⁴ From Sjoblad and Carlson memo (10/09/03)

Table 2. Summary of Dietary Exposure Normalized to Thymol

| U.S. Subpopulation | Foodstuff Residue/Honey Residue | | | | | Total of All Foodstuffs |
|----------------------------|---------------------------------|-------------------------|-------------------------------|--------------------------------|---------------------------------|----------------------------|
| | Honey (4.61 mg/kg) | Ice Cream (44 mg/kg) | Yellow Cake (6.5 mg/kg) | Cola Beverage (11 mg/kg) | Caramel Candy (9.4 mg/kg) | |
| General U.S. | 1 | 307.9 | 14.1 | 614.9 | 1.1 | 938.0 |
| Infant (6-11 mo) | 1 | 3.7E+09 | 6E+08 | 3.2E+09 | 2.0 | 7.5E+09 |
| Child (2 yr) | 1 | 417.7 | 19.6 | 746.2 | 7.3 | 1190.8 |
| Child (6 yr) | 1 | 158.7 | 6.6 | 113.5 | 0.03 | 278.8 |
| Child (10 yr) | 1 | 838.5 | 8.6 | 646.2 | 0.9 | 1494.2 |
| Female Juvenile (14-16 yr) | 1 | 662.4 | 22.7 | 1516.8 | 3.0 | 2204.9 |
| Male Juvenile (14-16 yr) | 1 | 973.6 | 30.2 | 2845.7 | 0.9 | 3850.4 |
| Female Adult (25-30 yr) | 1 | 543.1 | 12.0 | 1735.1 | 0.9 | 2291.1 |
| Male Adult (25-30 yr) | 1 | 364.0 | 11.8 | 1624.7 | 0.1 | 2000.6 |
| Female Adult (40-45 yr) | 1 | 270.7 | 18.1 | 533.8 | 0.3 | 822.9 |
| Male Adult (40-45 yr) | 1 | 868.5 | 66.7 | 2033.8 | 21.2 | 2990.2 |
| Female Adult (60-65 yr) | 1 | 444.5 | 35.3 | 453.9 | 9.3E+09 | 933.7 |
| Male Adult (60-65 yr) | 1 | 123.2 | 6.7 | 120.7 | 0.03 | 250.6 |
| Female Adult (70 yr) | 1 | 179.0 | 9.8 | 98.7 | 0.3 | 287.8 |
| Male Adult (70 yr) | 1 | 207.6 | 10.8 | 135.5 | 0.01 | 353.9 |

Drinking Water:

Exposure to thymol residues in drinking water is not expected, since the application of Apiguard occurs within the hive box. Exposure to the outside environment is thereby limited.

2) Non-dietary Exposure:

Non-dietary exposure to thymol is not expected, since the application of Apiguard occurs within the hive box. Exposure to the outside environment is thereby limited. In addition, occupational dermal and inhalation exposure will be mitigated by PPE (waterproof gloves, eye protection, long pants, and a long-sleeved shirt) and the physical nature of Apiguard (gel in a

container). Since the product is gel-based and physically constrained in a tray, the effects of spray drift do not have to be considered.

E. CUMULATIVE EXPOSURE:

Thymol has a novel mode of cellular action (GABA_A receptor, sodium, potassium, and calcium channel modulator) compared to other currently registered active ingredients. In addition, there is no indication that toxic effects of thymol would be cumulative. Section 408(b)(2)(D)(v) of the FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity."

EPA does not have, at this time, available data to determine whether thymol has a common mechanism of toxicity with other substances. Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to thymol and any other substances and thymol does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that thymol has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at <http://www.epa.gov/pesticides/cumulative/>.

F. SAFETY DETERMINATION:

1) US Population:

Dietary exposure to thymol residues in honey resulting from the use of Apiguard do not add significantly to the current dietary exposure of thymol from various foodstuffs. The dietary exposure from Apiguard-derived thymol in honey for all assessed populations is 250 - 7.5E+09 times *less* than the total dietary exposure from other foods such as ice cream, yellow cake, cola beverages, and caramel candies. This conclusion is based on comparative calculations involving FDA ingestion rates for various foods, EPA body weights, and residue concentrations from the literature and registrant studies. In addition, the maximum dietary exposure to thymol in honey (dark grey; 0.000232 mg/kg) is 2.76E+06 times lower than that from the most toxic acute toxicity study (LD₅₀ of 640mg/kg in mice). It is therefore adequate to conclude that there is reasonable certainty that no dietary harm will come from the use of Apiguard (thymol) in beehives at the label proposed rate.

Consumption of thymol residues in water from this application is also not expected to result in unacceptable risks, since the chemical is contained in a dispenser tray, is applied only to the inside of bee hives, and is rapidly volatilized or redistributed (2-4 weeks per package). Migration to potable water resources, therefore, is highly improbable.

Conservative assumptions when doing comparative risk calculations to food-based

thymol assured that the risks from Apiguard-derived thymol in food or water were not underestimated.

2) Infants and Children

FFDCA section 408 provides that EPA shall apply an additional tenfold margin of exposure (safety) for infants and children in the case of threshold effects to account for pre-natal and post-natal toxicity and the completeness of the data base unless the EPA determines that a different margin of exposure (safety) will be safe for infants and children.

A margin of exposure approach was not able to be performed in this instances because information on the subchronic or chronic effects of thymol were not requested. Even so, EPA concluded that the toxicology database was complete for FQPA purposes and that there were no residual uncertainties for pre-/post-natal toxicity resulting from thymol. This conclusion is based on the following:

Hazard: There is no evidence of unique fetal susceptibility in any of the acceptable studies presented. Genotoxicity and mutagenicity tests were negative by a weight of evidence. Adverse mammalian developmental effects were absent as well from acceptable studies. In addition, adverse effects from the consumption of food containing thymol have not been reported in the public literature.

Exposure: Differential pre- and post-natal exposures have also been considered in light of FQPA requirements. Both pre- and post-natal populations have been taken into account when estimating dietary exposure. Assessed populations included 6-11 month old infants and 2, 6, and 10 years old children. Additional assessed populations for both genders (male and female) included juveniles (14-16 years old), young adults (25-30 years old), adults (40-45 years old), and elder adults (60-65 and 70 years old). These populations bracketed and are thought to provide adequate representation for the sensitive subpopulation "females 13-49" years of age.

In summary, pre- and post-natal exposure has been considered either qualitatively or quantitatively in a comparative dietary risk assessments. Conservatism built into these exposure assessments suggest that special susceptibilities of these populations have been appropriately accounted for.

G. EFFECTS on the IMMUNE and ENDOCRINE SYSTEMS:

No studies illustrating thymol-induced immune and endocrine toxicity were submitted by the registrant. Information submitted in a peer-reviewed publication (MRID 46282803; Hagan et al., 1967), however, describe immunological endpoints in relation to short-term and chronic dosing. In the study, no effects were seen in the thymus, spleen, lymph nodes, white cell counts, red cell counts, hemoglobin counts, or hematocrits following the dosing of rats with 1000 or 10000mg/kg of food grade thymol for 19 weeks. The provided information is acceptable.

EPA is required under the Federal Food Drug and Cosmetic Act (FFDCA), as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect

produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." Following the recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there was scientific bases for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA has authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

When the appropriate screening and/or testing protocols being considered under the Agency's EDSP have been developed, thymol may be subjected to additional screening and/or testing to better characterize effects related to endocrine disruption.

H. EXISTING TOLERANCES:

In the US, residues of thymol currently have a time-limited exemption from the requirement of a tolerance in or on honey or honeycomb when used in conjunction with section 18 emergency exemptions granted by EPA. Time-limited exemptions expire and are revoked on June 30th of 2005.

Thymol is currently approved by the U.S. Food and Drug Administration (FDA) as a synthetic flavoring substance for the direct addition to food for human consumption (FDA, 21CFR §172.515) and as a preservative and indirect food additive of adhesives (FDA, 21CFR §175.105). The source plant of thymol, thyme or wild and creeping thyme (*Thymus vulgaris* L., or *Thymus serpyllum* L.) is also currently acknowledged by FDA as a spice, natural oil, oleoresin, or natural extract that is generally recognized as safe (21CFR §182.10, 21CFR §182.20). Although constituents vary depending on species and cultivation method, thyme can be comprised of up to 60% thymol (De Vincenzi et al., 1991).

In contrast to food uses, FDA has determined that there is inadequate evidence to establish the (general recognition of) safety and effectiveness for thymol when used as a topical acne treatment; a nasal decongestant; a dandruff/seborrheic dermatitis/psoriasis treatment; an external analgesic or anesthetic; a fever blister and cold sore treatment; a poison ivy, oak, and sumac treatment; an oral health care treatment; a skin protectant-astringent treatment, and a topical antifungal treatment (FDA, 21CFR §310.545). FDA has also banned it from use as an ingredient in smoking deterrent products (FDA, 21CFR §310.544), and an over the counter treatment for boils (FDA, 21CFR §310.531) because these uses have not been shown to be safe and effective.

I. INTERNATIONAL TOLERANCES:

Regulatory limits have been set for thymol in other countries. The Swiss Federal Department of the Interior has set a tolerance value for thymol in honey as an antiparasitic agent (0.8 mg/kg; pharmacological substance active in nutrition or therapeutic application; 817.021.23). This tolerance was derived to prevent exceedance of the taste threshold for thymol

in honey (1.1 - 1.3 mg/kg; Bogdanov et al., 1999). The European Agency for the Evaluation of Medicinal Products (Committee for Veterinary Medicinal Products; EMEA/MRL/075/96) has also established an indication of use for thymol at 10 mg per animal (horse, swine, cattle, sheep, and dogs) for up to 5 days for the treatment of respiratory tract ailments. Residues of thymol in treated animals and products were not thought to be of toxicological concerns for humans in this case. The Committee of Experts on Flavouring Substances of the Council of Europe has also established a limit for thymol inclusion in food at 50 mg/kg and beverages at 10 mg/kg (2002). Thymol is currently exempted from a food MRL in New Zealand.